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(71) Applicant (for all designated States except US):
SYNAMEM CORPORATION [US/US]; 863B Mit-
ten Road, Burlingame, CA 94010 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): YAMAZAKI, Vic-
toria [US/US]; 77 Dow Place, #907, San Francisco, CA

94107 (US). SIRENKO, Oksana [US/US]; 421 37th Av-
enue, San Mateo, CA 94403 (US). GROVES, John, T.
[US/US]; 750 North Shoreline, Mountain View, CA 94043
(US).

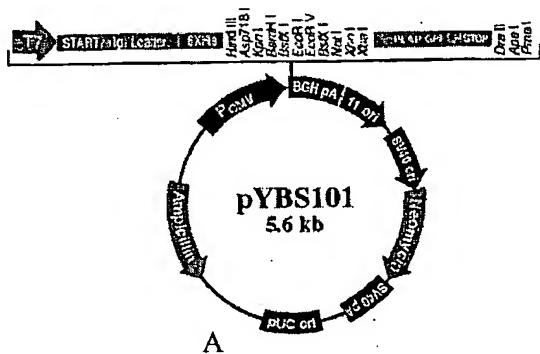
(74) Agents: MAHONEY, Jacqueline, F. et al.; Perkins Coie
LLP, P.O. Box 2168, Menlo Park, CA 94026 (US).

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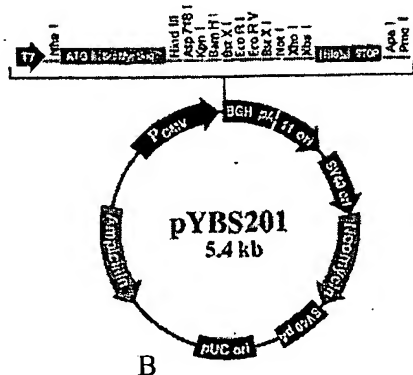
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[Continued on next page]

(54) Title: METHOD FOR GENERATING TETHERED PROTEINS



(57) Abstract: The present invention relates to a novel
method of generating tethered extracellular or intracellular
domains of transmembrane proteins using expression
vectors. The invention also provides the expression vectors
for use in the world.



INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2005/004497

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/62 C12N15/85 C07K14/705

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, CAB Data, Sequence Search, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 03/089649 A (OXFORD BIOMEDICA LIMITED; KINGSMAN, SUSAN; CARROLL, MILES; MYERS, KEV) 30 October 2003 (2003-10-30)	1,6,8,10
Y	figures 1-4; examples 1,3	2-5,9
X	WO 96/41865 A (ARIAD GENE THERAPEUTICS, INC; CLACKSON, TIMOTHY; HOLT, DENNIS, A; GILM) 27 December 1996 (1996-12-27) page 100, line 16 - page 101, line 6; figure 12A	1,6,7, 11,12
X	WO 94/18317 A (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIO; PRESIDENT AND FELL) 18 August 1994 (1994-08-18) page 54, line 24 - page 55, line 11; figure 21B	1,6,7, 11,12
	----- -/-	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

19 May 2005

Date of mailing of the international search report

01/06/2005

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Hornig, H

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US2005/004497

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>WO 89/01041 A (GENENTECH, INC) 9 February 1989 (1989-02-09) the whole document</p> <p>-----</p>	2-5,9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2005/004497

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-7 (as far as in vivo methods are concerned) are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2005/004497

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 03089649	A	30-10-2003	AU 2003227866 A1 WO 03089649 A1	03-11-2003 30-10-2003
WO 9641865	A	27-12-1996	AU 714904 B2 AU 6270696 A CA 2219080 A1 EP 0833894 A1 JP 2002514893 T US 6187757 B1 WO 9641865 A1 US 2003206891 A1 US 6506379 B1 US 2004082515 A1 US 2002107189 A1	13-01-2000 09-01-1997 27-12-1996 08-04-1998 21-05-2002 13-02-2001 27-12-1996 06-11-2003 14-01-2003 29-04-2004 08-08-2002
WO 9418317	A	18-08-1994	AU 690898 B2 AU 6240394 A AU 7880798 A CA 2155728 A1 CN 1119876 A CZ 9502061 A3 EP 0804561 A1 FI 953812 A HU 73101 A2 JP 8510896 T PL 310327 A1 US 2004024725 A1 US 6165787 A US 6011018 A US 6054436 A WO 9418317 A1 US 6063625 A US 6046047 A US 6043082 A US 6140120 A US 5834266 A US 6316418 B1 US 5869337 A US 5871753 A US 5830462 A US 5994313 A US 2002173474 A1	07-05-1998 29-08-1994 08-10-1998 18-08-1994 03-04-1996 17-04-1996 05-11-1997 11-08-1995 28-06-1996 19-11-1996 11-12-1995 05-02-2004 26-12-2000 04-01-2000 25-04-2000 18-08-1994 16-05-2000 04-04-2000 28-03-2000 31-10-2000 10-11-1998 13-11-2001 09-02-1999 16-02-1999 03-11-1998 30-11-1999 21-11-2002
WO 8901041	A	09-02-1989	US 5109113 A AU 629517 B2 AU 2308788 A DE 3854328 D1 DE 3854328 T2 EP 0371999 A1 IL 87366 A JP 2504467 T JP 2935709 B2 US 6632634 B1 US 5374548 A WO 8901041 A1 US 5763224 A US 5264357 A	28-04-1992 08-10-1992 01-03-1989 21-09-1995 28-03-1996 13-06-1990 18-08-1993 20-12-1990 16-08-1999 14-10-2003 20-12-1994 09-02-1989 09-06-1998 23-11-1993

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220



WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (PCT Rule 43bis.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.
PCT/US2005/004497

International filing date (day/month/year)
09.02.2005

Priority date (day/month/year)
09.02.2004

International Patent Classification (IPC) or both national classification and IPC
C12N15/62, C12N15/85, C07K14/705

Applicant
SYNEMEM CORPORATION

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:



European Patent Office - P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk - Pays Bas
Tel. +31 70 340 - 2040 Tx: 31 651 epo nl
Fax: +31 70 340 - 3016

Authorized Officer

Hornig, H

Telephone No. +31 70 340-2620



**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US2005/004497

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
 - ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).
2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
 - a. type of material:
 - ☐ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material:
 - ☐ in written format
 - ☐ in computer readable form
 - c. time of filing/furnishing:
 - ☐ contained in the international application as filed.
 - ☐ filed together with the international application in computer readable form.
 - ☐ furnished subsequently to this Authority for the purposes of search.
3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

Box No. II Priority

1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43bis.1 and 64.1) is the claimed priority date.
2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43bis.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.
3. Additional observations, if necessary:

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US2005/004497

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application,
- ☒ claims Nos. 1-7

because:

- ☒ the said international application, or the said claims Nos. 1-7 relate to the following subject matter which does not require an international preliminary examination (*specify*):

see separate sheet

- ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
- ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
- ☐ no international search report has been established for the whole application or for said claims Nos.
- ☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:
 - the written form ☐ has not been furnished
 - ☐ does not comply with the standard
 - the computer readable form ☐ has not been furnished
 - ☐ does not comply with the standard
- ☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-bis of the Administrative Instructions.
- ☐ See separate sheet for further details

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/US2005/004497

Box No. V Reasoned statement under Rule 43bis.1(a)(I) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	2-5,9
	No: Claims	1,6-8,10-12
Inventive step (IS)	Yes: Claims	
	No: Claims	1-12
Industrial applicability (IA)	Yes: Claims	8-12
	No: Claims	1-7

2. Citations and explanations

see separate sheet

Re Item III.

1.1 Claims 1-7 relate to subject-matter considered by this Authority to be covered by the provision of Rule 67.1 (iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Art. 34(4)(a)(I) PCT).

Re Item V.

1 Reference is made to the following documents:

D1 : WO 03/089649 A (OXFORD BIOMEDICA LIMITED; KINGSMAN, SUSAN;
CARROLL, MILES; MYERS, KEV) 30 October 2003 (2003-10-30)

D2 : WO 96/41865 A (ARIAD GENE THERAPEUTICS, INC; CLACKSON, TIMOTHY;
HOLT, DENNIS, A; GILM) 27 December 1996 (1996-12-27)

D3 : WO 94/18317 A (THE BOARD OF TRUSTEES OF THE LELAND STANFORD
JUNIO; PRESIDENT AND FELL) 18 August 1994 (1994-08-18)

2 INDEPENDENT CLAIMS 1, 8 and 11

2.1 The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1 and 8 is not new in the sense of Article 33(2) PCT. Document D1 discloses an expression vector comprising an amino-terminal tag sequence and a signal sequence operably linked to a nucleotide sequence of interest, where the amino-terminal tag sequence is inserted between the signal sequence and the nucleotide sequence of interest which is a tumour associated antigen (TAA 5T4), characterised as

membrane protein. Constructs for a membrane-bound protein are made which were cloned in pIRES-STAR vector and transiently transfected into CHO cells and expression of h5T4 detected by immuno-staining of fixed cells with an anti-myc antibody (Examples 1-3, Fig. 1-4).

Therefore, a method of generating tethered extracellular domains of transmembrane proteins comprising: (a) preparing an expression vector comprising a 5' signal sequence, a purification epitope tag, a sequence coding for the extracellular domain of a membrane protein and a 3' anchor sequence, and transfecting mammalian cells with said expression vector to generate anchor tethered protein targeted to the extracellular domain of a plasma membrane does already exists.

2.2 The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1 and 11 is not new in the sense of Article 33(2) PCT. Document D2 discloses configurations for biological switches and provides new methods and materials useful for regulating biological events in animal cells. The invention involves recombinant DNA constructs comprising DNA sequences derived from sequences encoding the proteins FRAP, Tor1, Tor2 and other proteins capable of binding to FKBP:rapamycin. The products can be used for regulating biological events such as gene transcription and activation of an intracellular signal transduction pathway. Furthermore D2 describes the cloning of the cytoplasmic domain of a receptor tyrosine kinase into the XbaI site of pCMFR series or pCMF series of vectors and the cotransfection into Cos-1 cells by lipofection (page 100, lines 16-page 101, lines 27).

The plasmids pCMF11/2/3.HA respectively pCMFR1/2/3.Flag have the following features: a myristoylation domain and a HA, respectively a Flag epitope tag and a XbaI site in between, into which the cytoplasmic domain of a receptor protein was cloned.

Therefore, a method of generating tethered extracellular domains of transmembrane proteins comprising: (a) preparing an expression vector comprising a 5' myristoylation encoding sequence, a sequence coding for the intracellular domain of a membrane protein and a 3' purification epitope tag, and transfecting mammalian cells with said expression vector to generate myristoylated tethered protein targeted to the intracellular domain of a plasma membrane does already exists.

2.2 The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1 and 11 is not new in the sense of Article 33(2) PCT. Document D3 developed a general procedure for the regulated (inducible) dimerization or oligomerization of intracellular proteins. A DNA construct is disclosed which encodes a chimeric protein comprising (a) at least one receptor domain, capable of binding to a selected ligand, fused to (b) a heterologous additional protein domain capable of initiating a biological process upon exposure to the ligand, the ligand being capable of binding to 2 or more chimeric protein molecules. A chimeric cDNA has been prepared consisting of three FKBP12 domains fused to the cytoplasmic signalling domain of the Fas antigen and stably transfected as MC3FE construct (M=myristoylation domain of Scr, C= cyclophilin domain, F= cytoplasmic tail of Fas, E= influenza haemagglutinin (flu) epitope tag) in Jurkat T cells.

Therefore, a method of generating tethered extracellular domains of transmembrane proteins comprising: (a) preparing an expression vector comprising a 5' myristoylation encoding sequence, a sequence coding for the intracellular domain of a membrane protein and a 3' purification epitope tag, and transfecting mammalian cells with said expression vector to generate myristoylated tethered protein targeted to the intracellular domain of a plasma membrane does already exist.

3 DEPENDENT CLAIMS 2-5 AND 9

Dependent claims 2-5 and 9 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of novelty and/or inventive step (Article 33(2) and (3) PCT).